1954

Some pathological changes in trout organs caused by certain strains of the genera Cytophaga and Pseudomonas

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SOME PATHOLOGICAL CHANGES IN TROUT ORGANS CAUSED BY CERTAIN STRAINS OF THE GENERA CYTOPHAGA AND PSEUDOMONAS

by

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B. A. Montana State University, 1953

Presented in partial fulfillment
of the requirements for the degree of
Master of Arts

MONTANA STATE UNIVERSITY
1954

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Aug 17 1954
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To My Wife
patient and devoted

ACKNOWLEDGMENTS

The author wishes to express his deepest appreciation to Mr. E. E. Jeffers for his suggestions and helpful guidance throughout the course of the study. Gratitude is accorded Dr. D. M. Hetler for his suggestions and assistance. I wish to thank Dr. George Weisel and Mr. Jack Bailey for their aid in the study preparations, and my sincere appreciation to Mrs. Pauline Niemeyer for the media preparations necessary for the study.

J. W. J.

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INTRODUCTION

Trout diseases received some attention in the eighteen hundreds. Fabre-Domergue (1890) described a tumor-like lesion in salmonid fishes and stated that the lesion was full of bacteria. He made cultures on fish gelatin. The gelatin was liquified and assumed a green flourescence. Charrin (10) isolated several strains of bacteria from diseased fish. One of these strains proved to be pathogenic. This pathogen was a motile bacterium which grew at 20° C. but not at 37° C. Fish became infected from water contaminated with the microorganism.

Trout diseases in the United States assumed major importance when attempts were made at artificial propagation. In 1885 Green (6) described an epidemic affecting Rainbow trout at McCloud River Station. Bean (1) in 1894 reported a severe epidemic among Brook trout in Michigan.

Early reports such as these lacked informative facts due to the embryonic status of the study and uninformed workers in the field. It was not until the early nineteen hundreds that work of a more practical value was done. Among these studies a publication by Gorham (5) described a gas-bubble disease and its cause. He considered its chief manifestation exophthalmia, or popeye, to be the result of an excess of dissolved air in the blood. Manning (9) attributed an exophthalmia to a myxospordian parasite which attacked the lumen of the kidney tubules. The eye lesion was said to be the result of an accumulation of serous fluids in the body

cavities.

One of the first references to a pathological condition of the gill tissues of salmonid fishes was that of Osburn (12). He observed a marked proliferation of gill epithelium and assumed the resulting manifestation to be due to continual irritation of the delicate gill tissue in the absence of the usual protection offered by the normal operculum. Davis (2) stated the causative organism in gill disease to be a filamentous rod-shaped bacterium usually found in great abundance in typical cases of the disease. This organism was not a tissue invader and its chief damage to the host appeared to be the irritation produced by the surface growth on the delicate gill epithelium. Ordal and Rucher (11) studying bacterial gill disease, consistently isolated myxobacteria from gills of infected fish. Wolf (13) working in this phase of pathology had reported failure to transmit the disease to healthy fish by contact, feeding infected material or by introduction of pure cultures into water.

Kidney infections have manifested themselves in many species of trout. Rucker and Burrows reported a kidney infection of blueback salmon caused by a gram-negative, nonmotile, rod shaped bacterium. Damage to kidney tissue in a generalized disease known as "Furunculosis" was described by Fish (3) in 1937. The organ became necrotic and semi-fluid and the bacteria collected in the kidney in great numbers, especially in the glomeruli where they could be seen on sectioning.

Reports of investigations indicate that many hatcheries in the United States have been plagued with trout diseases of a generalized nature. Montana's fish hatcheries are no exception. Davis, in 1926, reported infections of gill filaments by pathogenic rod-like organisms.

Jeffers (unpublished) has isolated many strains of Pseudomonas and myxobacteria from lesions and degenerate tissue from diseased trout. His findings have suggested an interrelationship between Pseudomonas and myxobacteria. Field studies on the occurrence of Furunculosis among Loch Leven trout in Lake Madison, Montana were undertaken during 1951 (4). Cultures of unknown species were made from the kidneys of adult fish by Dr. C. J. Brown of Montana State College. Hatcherymen throughout the state have reported several epidemics in trout populations, though no etiological agent was isolated.

As a result of this confusion the author has chosen as the field of study, certain pathological changes in organs most commonly afflicted, the gill filaments, the peritoneal cavity, the kidneys, the ophthalmic and brain areas. These three areas were chosen because of their apparent greater susceptibility to infections.

As these diseases occur in nature and have not been attributed solely to human activity, it appears safe to assume that some of the normal aquatic flora may be considered capable of initiating and maintaining these infections. With this in mind, the author has chosen two groups of organisms, strains of the genus Pseudomonas and certain myxobacteria.

CHAPTER I

PROCEDURE

Types of Trout Used

Approximately five hundred fingerling Rainbow trout (Salmo gairdnerii irideus) were obtained from the Emmigrant hatchery near Livingston, Montana. A similar number of fingerling Cutthroat trout (Salmo Clarkii) were obtained from the Arlee hatchery.

As a preliminary autopsy revealed no diseased trout, the trout from both hatcheries were considered normal.

Types of Organisms Used

Several organisms were isolated from water samples from the Emmigrant hatchery ponds. The water samples were obtained from the upper and lower portions of several ponds. From the water samples from the upper portions of the ponds, three cultures of the genus Cytophaga and two cultures of the genus Pseudomonas were selected on the basis of pigmentation, colony morphology and cell morphology. These cultures were lettered A, B, C, etc. for the area from which they were isolated. From lower pond samples three cultures of genus Pseudomonas were selected in a similar manner.

From kidney isolations, four strains of the genus Cytophaga and one strain of Pseudomonas were selected. No isolations were made from

gill or brain tissue.

To each strain a letter was assigned for identification purposes. Cultures lettered A, B, D, A', D', E', M, N, O, and P were members of the genus Cytophaga. Organisms lettered C, E, B', C', and Q were members of the genus Pseudomonas.

Materials

A. Media used included the following:

1. Tryptone agar as described by Ordal and Rucker (11). This medium was modified to include 0.05% K_2HPO_4 and 0.1% yeast extract. The final pH was 7.2.
2. Tryptone broth which included the same materials as the above mentioned agar minus the agar.

B. Special equipment included the following:

1. An aquarium (15" by 18" by 48") of glass and wrought iron construction. Its capacity was forty gallons.
2. Plastic filter (8" by 4" by 6") containing activated carbon particles for dechlorination.
3. A Marco air pump of 115 volts and 60 cycles with two outlets.
4. Histological equipment including: fixatives, alcohols, clearing agents, paraffin, microtome, warming plate, oven, clean slides and various stains.

C. Miscellaneous materials used:

1. Sterile tuberculin syringes and needles.
2. Sterile physiological saline.

3. Crystalline urethane.

4. Dietary factors (horse heart, vitamin A).

5. Colorimeter, thermometers and various chemicals.

6. Glass tubing and rubber hose.

Methods of Maintaining Trout

Trout maintenance under laboratory conditions was found to be a problem in itself. The aquarium water supply was a critical factor in effectually perpetuating a satisfactory environment. A constant influx of fresh dechlorinated water was kept running into the tank. This was accomplished with a series of rubber and plastic tubes connected to a water faucet, passing to a carbon filter and finally opening into the aquarium. An outlet was constructed as an overflow. Water temperature was controlled by a decrease or increase of water flow through the system as the situation demanded.

The diet consisted of finely minced horse heart fed four times daily. The amount fed depended upon the amount consumed at feeding time. It was found that illness, high temperatures, and a low pH reduced appetites considerably. Waste feed accumulation on the tank bottom was avoided as much as possible.

A Marco air pump was employed to maintain a proper oxygen-nitrogen balance. This balance had to be maintained at all times to prevent asphyxiation from gas embolism in the gill filaments, or heart.

Exposure to direct sunlight was avoided.

Measures were taken to reduce slime formation on the tank walls by drainage of the aquarium followed by a cleansing with a rag and alcohol.

Experimental Control of Certain Other Factors

It was theorized that the experimental control of critical environmental factors would render the trout more susceptible to infection. Some conditions were maintained at a level below optimum for good trout growth. It was hoped that in governing these factors a resulting decrease in resistance would facilitate and hasten the prodromal stage. These controls are discussed in detail below.

Some dietary factors were controlled. There is a general belief that dietary factors might govern the extent and frequency of trout diseases. Among these factors the vitamins assume importance. Horse heart, stripped of the fatty tissue, fulfilled the requirements adequately as a minimal vitamin-A free diet. C and D also existed in small amounts in this diet. No supplements were added such as vitamins, minerals, etc. Water temperature was expected to play an important role in experimental trout maintenance. According to Jack Bailey, Montana Fish Biologist, the temperature which is optimum for trout in his area is 10-12° C. Temperatures above 18.3° C. are considered improper for trout species. The temperature for optimum myxobacteria and Pseudomonas growth is from 20°-25° C. and not above 30° C. for the strains isolated in his area. To facilitate the proper temperatures for both fish and bacteria, the water was held at 16° C. with occasional slight variation in either direction.

The pH of the water was not altered significantly. A continual check was made to prevent too great a lowering of pH. The pH value was kept at a constant 6.8, a relatively easy matter due to accumulated excretions lowering the reading. If the pH lowered excessively a 10% solution of sodium thiosulphate was effective in raising the reading.

Methods of Initiating Diseases

Method I

The mode of entry of pathogenic bacteria into trout has never been successfully determined. The most obvious route would seem to be the gill filaments. To artifically induce an infection of the gill and facilitate the entry of organisms into the body two procedures were attempted.

Procedure I: Fingerling Rainbow trout were partially anesthetized in a 2% solution of urethane (two parts urethane to 98 parts distilled water). Forty-eight hour slant cultures of the fifteen organisms were washed down with 10 cc of sterile physiological saline and further diluted to 1:100. Each culture was quickly but gently swabbed over the gill filaments with a sterile cotton swab. Each culture was streaked over five pairs of gills. Five trout were likewise swabbed with a sterile cotton swab for controls. A portion of one of the fins was clipped or punched for identification. Upon death, the trout were examined, the external afflictions recorded, and the tissue placed in preservative for pathological study. Trout which had not died after thirty days were sacrificed and examined. The findings were recorded.

Procedure II: This method utilized natural trout-organism contact. This was done to illustrate the ability of microorganisms to infect trout by contact in water. To accomplish this, mass innoculations of all fifteen strains of microorganisms were made into the aquarium. One 4 mm loopful of a 48 hour tryptone broth culture was distributed over the surface of the water followed by agitation with a glass rod to assure wide dispersion of the organisms. Each culture was successively

innoculated into the water in this manner. A single innoculation of each culture was considered sufficient. After death, findings were recorded. A period of thirty days was permitted before the trout were sacrificed.

Method II

In the second aspect of the initiation of disease, direct innoculation of organs with 48 hour cultures of myxobacteria and strains of Pseudomonas were made. One diluted culture was innoculated into each trout. It was hoped that this would give a clear pathological picture of each organ and therefore possibly aid in identifying the species of organism causing the infection.

The brain area: Each trout was anesthetized with a 2% solution of urethane. A one cc tuberculin syringe with a 28 guage needle was used to inject a 1:200 diluted suspension of the organism in 0.01 cc amounts into the area immediately postero-lateral to the brain. Each strain of microorganism was innoculated into three fish. As a control, three fish were injected with 0.01 cc of physiological saline held at 15° C. into the same area. Each trout was fin-marked for identification of cultures used for injection. Upon death innoculated fish were checked for organisms and gross pathological change which may have occurred as a result of brain injections. The findings were recorded and the tissue placed in preservative (70% alcohol) for histopathological study.

The ophthalmic area: The procedure employed in ophthalmic injections was similar to that of brain injections. An area medio-lateral to the eye and antero-lateral to the brain was innoculated with selected strains of microorganisms. Each trout was fin-marked for identification.

External features were recorded and the tissue placed in preservation for further study.

The peritoneal cavity: Trout were anesthetized with urethane and injected intraperitoneally with 0.02 cc amounts using a 1:100 dilution of the organism. Controls were injected with sterile physiological saline in 0.02 cc amounts. Each trout was fin-marked for identification. At death gross features of organs were recorded and the tissue preserved. If death had not occurred after thirty days, the fish were sacrificed, inspected, and the findings recorded.

The kidney: Difficulty was encountered in intrakidney injections. To aid innoculations, an intense light was utilized to outline the kidney in the live anesthetized trout. The trout were then injected with 0.02 cc amounts of a 1:200 suspension of the organism with a 1 cc tuberculin syringe. Controls were injected with a similar amount of sterile saline. Five trout were used for each organism. After death bacterial isolations were made, the organs were checked and the tissue preserved for further study. Trout not dead after thirty days were sacrificed and inspected.

Another method used for kidney injections employed a micro-injection unit constructed from glass tubing as described by Guyer (7). Reasonably accurate injections could be made with this apparatus. It consisted of a glass bulb tapered to a fine tip. A stand was constructed to hold this bulb in a steady position for injections involving extreme care and precision. Fluid intake was accomplished by heating the empty bulb, placing in the desired fluid for injection, and thus the vacuum drawing the fluid into the bulb. To eject the fluid, a slight touch of heat to the top of the bulb forced out the material rapidly. A 1:500

dilution of each microorganism was prepared by washing down the culture from the medium with 10 cc of sterile .086% physiological saline. From the resulting 1:10 suspension of the organisms, the required dilution was made. This dilution was injected into anesthetized trout which were held gently but firmly in molding clay. A strong beam of light was passed through the fish to outline the kidney and the fine point of the microinjection unit was passed into the organ. The amounts injected varied slightly due to inability to control the liquid stream.

Procedure in histology

After a preliminary check for external features the organs were placed in a preservative (70% alcohol) in preparation for histopathology. The technique used was described by Guyer (7) with modifications in the staining technique by Ziegler (14). It was found, however, that the paraffin infiltration time length extended from 24-36 hours in 52° C. paraffin to permit a uniform infiltration of all organs. In the staining technique the quadruple staining method was utilized more frequently. Wright's stain was used for staining of blood samples. Giemsa's and Gram stains were used for staining of organisms on slides and attempts were made to stain the organisms in the tissue with the Gram stain.

CHAPTER II

RESULTS

Symptomology

The symptoms of the infections induced were stereotype in appearance with the exception of the cartilage deterioration which occurred in brain-area injected trout. Upon the onset of the disease an apparent lack of appetite was evident followed by a change in color. The color changes involved a transition from a lighter to a darker shade. Shortly the trout began to move nearer the surface as if to elude pressure or balance a gaseous condition. Vigor decreased rapidly until the trout exhibited little motion. Gradually they sank to the bottom and with extreme difficulty attempted to maintain equilibrium. Eventually the trout turned on their sides with gills vigorously active and finally died.

The symptoms involved in cartilage degeneration differed to the extent of a sudden onset of an erratic behavior. This included wild surges back and forth in the tank followed by twists and rolls as if in convulsions. After a period of four to five hours they died in lessening convulsive spasms.

Bacteriology of the blood and organs

Several strains of Cytophaga and Pseudomonas were isolated from

the blood in cases of induced gill and kidney infections. These isolations were identical in morphology, pigmentation, and fructation with those causing the infection.

Isolations were made from infected organs. These corresponded in morphology and pigmentation to those organisms injected previously into the organ. However, in several cases a change in pigmentation was noted, possibly due to parasitic existence in the trout.

CHART I

RESULTS OF MYXOBACTERIAL-PSEUDOMONAS*
INFECTION OF THE GILL

Org.	Strain of Org.	Inject. Infect.	Pathology of gills	Pathology of other organs
A	myxo	5/0	None	None
B	myxo	5/4	Grayish mucous mass over outer half of gill. Epithelial proliferation, slight clubbing of lamellae.	Slight cartilage degeneration around brain. Lymphocytic infiltration of kidney.
C	Pseudo	5/2	Mucous mass over outer half of gill filaments. Gills apparently irritated.	None
D	myxo	5/4	Irritated. No proliferation. Transparent mucous mass over entire gill. No lesions.	None
E	Pseudo	5/0	None	None

A'	myxo	5/5	Transparent mucous mass over gill. No epithelial proliferation. No lesions	None
B'	Pseudo	5/0	None	None
C'	Pseudo	5/1	Small amount of mucous on outer curves of gill arches.	None
D'	myxo	5/0	None	None
E'	myxo	5/2	Mucous mass on outer third of gills. No proliferation of tissue.	None
M	myxo	5/5	Transparent mucous mass over entire gill. Some tissue proliferation. No lesions.	Uriniferous tubules degenerated. Slight infiltration of lymphocytes.
N	myxo	5/4	Grayish mucous mass on outer half of gills. No tissue proliferation.	Slight lymphocytic infiltration.
O	myxo	5/3	Mucous mass on outer curves of gill arches. No tissue proliferation.	None
P	myxo	5/5	Grayish mucous mass on gills. No proliferation of tissue.	Columnare cells of tubules reduced in size.
Q	Pseudo	5/3	Grayish mucous mass over outer half of gill. No gill proliferation	Slight lymphocytic infiltration.

CONTROLS	None	None

Note: For clarification of lettered organisms see page 4.
* myxo - myxobacteria
Pseudo - Pseudomonas

The gills

The data used to set up Chart I was obtained from those trout actually swabbed with microorganisms. The lack of a means of positively identifying the organisms employed prevented an accurate estimate of the results from the mass innoculation phase.

Gross pathology of gill: The histo-pathological progress of gill disease in the trout infected appeared to start at the distal end of the gill filaments located along the outer curve of the gillarch. In the normal gill the blood stream is separated from the circulating water by the endothelium of the capillary and one layer of simple squamous epithelium. The epithelium in pathological stages proliferates at the distal ends of the lamelli and along the gill filaments. This "clubbing" effect of the lamelli was found in two cases in the study. In several cases a large amount of bacterial growth was noted occasionally accompanied with irritated gill epithelium. In all other cases the gill epithelium appeared normal.

CHART II

RESULTS OBTAINED FROM BRAIN AREA INNOCULATIONS WITH STRAINS OF MYXOBACTERIA AND PSEUDOMONAS*

Org.	Strain of Org.	Inject. Infect.	Pathology of area	Pathology of other organs
A	myxo	3/0	None	None
B	myxo	3/3	Cartilage degeneration. Brain apparently normal.	None
C	Pseudo	3/0	None	None
D	myxo	3/3	Cartilage degeneration.	None
E	Pseudo	3/0	None	None
A'	myxo	3/2	No apparent damage.	None
B'	Pseudo	3/0	None	None
C'	Pseudo	3/0	None	None
D'	myxo	3/0	None	None
E'	myxo	3/0	None	None
M	myxo	3/3	Cartilage heavily attacked.	Slight kidney tubule degeneration.
N	myxo	3/3	None	Heavy kidney damage. Tubules disrupted. Interstitial tissue.
O	myxo	3/0	None	None
P	myxo	3/2	None	Slight kidney damage.
Q	Pseudo	3/0	None	None
CONTROLS		3/0	None	None

Note: For clarification of lettered organisms see page 4.
* myxo - myxobacteria
Pseudo - Pseudomonas

The brain area

The results obtained may be lacking in completeness due to inability to recognize pathological conditions of cortical tissue. However, cartilage degeneration was evident when it occurred. Isolations of organisms from degenerate cartilage supported their role as possible cartilage-attackers.

CHART III

RESULTS OF INTRAPERITONEAL INJECTION WITH MYXOBACTERIA AND PSEUDOMONAS*

Org.	Strain of Org.	Inject. Infect.	Pathology
A	myxo	3/2	Hemorrhaging of lower portion of intestine. Large amounts of serous fluids in body cavity. Pancreas and liver slightly degenerate.
B	myxo	3/2	Same as above
C	Pseudo	3/2	Large amounts of serous fluids present

Note: For clarification of lettered organisms see page 4.
* myxo - myxobacteria
Pseudo - Pseudomonas

The peritoneal cavity

As the data indicates only three cases of infection resulted from intraperitoneal injections. No other infections were observed. However, some organisms were isolated from the body cavity which showed no apparent damage.

The pathology of those fish injected revealed sites of hemorrhaging along the intestine. The pancreas and liver appeared to be

proteolyzed. Proteolytic action was apparent throughout the body cavity possibly giving rise to the large quantities of serous fluids.

The kidney

The infection of the kidney revealed several pathological pecularities. Of these the most common was the presence of large masses of lymphocytes in the interstitial tissue. These leukocytes were large, uninuclear cells staining dark blue with Wrights stain. Another pathological condition involved the uriniferous tubules. Normally these tubules are lined with high columnar cells with their surface to the lumen of the tubule. In several cases these cells were altered to such an extent they appeared to be cuboidal and even as simple squamous type epithelium. Often associated with this condition was the presence of lymphocytes. Two cases revealed another condition, one more marked than the other. This was the presence of sharp, clear crystals forming apparently from the tubule walls. In one case these crystals blocked the entire lumen of certain portions of the tubule. The tubule wall had clearly disintegrated.

Results of ophthalmic injections

No results were obtained from the direct injection of myxobacteria and Pseudomonas into the area medio-lateral to the eye. All fish were normal after a period of thirty days.

CHART IV

RESULTS OF KIDNEY INJECTIONS WITH STRAINS OF MYXOBACTERIA AND PSEUDOMONAS*

Org.	Strain of Org.	Injected / Infected	Pathology of kidney
A	myxo	5/5	Slight uniniferous tubular alterations. Large amount of lymphocytic infiltration.
B	myxo	5/5	Lymphocytic infiltration. Columnar cells lowered.
C	Pseudo	5/0	None
D	myxo	5/0	None
E	Pseudo	5/0	None
A'	myxo	5/0	None
B'	Pseudo	5/2	Slight amount of lymphocytic infiltration
C'	Pseudo	5/3	Some lymphocytes in interstitial tissue.
D'	myxo	5/0	None
E'	myxo	5/3	Slight tubule disruption.
M	myxo	5/5	Uriniferous tubules badly damaged. Lymphocytes. Small number of crystals in tubules.
N	myxo	5/5	Uriniferous tubules badly damaged. Lymphocytes. Crystals in large numbers blocking tubules.
O	myxo	5/4	Tubules damaged. Lymphocytes.
P	myxo	5/5	Large numbers of lymphocytes in tissue.
Q	Pseudo	5/5	Large numbers of lymphocytes in tissue.
CONTROL		5/1**	None

Note: For clarification of lettered organisms see page 4.
* myxo - myxobacteria
Pseudo - Pseudomonas
** Cause unknown

CHAPTER III

DISCUSSION

The foregoing study indicates that some strains of the genus Cytophaga and the genus Pseudomonas may act as etiological agents in trout disease.

The portal of entry for these organisms appears to be through the gill filaments. Although other possible routes may exist, the gills apparently have more advantages for bacterial introduction for two reasons: (1) the thin gill epithelium offers little obstruction for bacterial passage into the blood stream and (2) the continual exposure of gill surfaces to circulating organism-laden water.

Deaths resulting from gill infection may stem from three causes, the mechanical obstruction of the gill filaments by extreme bacterial growth, the proliferation or degenerative conditions impeding the vital exchange of gases between the water and blood stream, or a possible correlation may exist between gill infections resulting in manifestations of distant organs. Future studies may determine which cause is the more frequent but at the present all three must be considered when treatment is to be employed.

The failure to elicit an ophthalmic infection indicates the lesion not to be of direct bacterial affects. A more plausible theory

would be the end result of some inbalance of gases, pressure or the accumulation of deleterious toxins from some metabolic disorder or bacterial infection.

Many trout exhibited an erratic behavior when infected with some changes in external appearances. This would indicate an effect on brain or nervous tissue. The changing of color when parasitized might suggest an affliction of the autonomic nervous system. Certain essential endocrine organs may be affected during bacterial infections. The histopathological knowledge is lacking for these organs.

The failure to produce infections in the peritoneal cavity indicates a certain amount of tolerance or resistance toward infections by the trout in this area. Perhaps after a prolonged period trout resistance to these infections would increase.

The mesonephric kidneys of trout are, as the data indicates, sites of several pathological conditions. Their apparent susceptibility to bacterial attack suggests that their degeneration may result in immediate death. The organisms in the study attacked both the interstitial tissue and the uriniferous tubules. The mode of introduction of the bacteria into the kidney may occur through the blood. Organisms were isolated from blood during the infection period. The relationship of these organisms to the crystals is not clear. The crystals may be the result of a diet deficiency, probably a vitamin. The evidence, however, points to an association between the presence of Cytophaga and Pseudomonas, a vitamin-low diet, and the resulting formation of the crystals. The affect of the organisms upon the tubule epithelium perhaps alters or impedes the absorption of several compounds back into the blood

stream. Crystals may arise from the accumulation of some element normally absorbed in this disrupted area. The accompanying lack of the dietary factor might stimulate the crystal formation.

The data suggests a possible route of migration of these organisms upon contacting the gill epithelium. The passage of organisms through the gill epithelium into the capillaries of the aortic arches demonstrates itself from isolations of these organisms from blood samples of gill infected trout. The migration would proceed to the dorsal aorta which would serve as a mode of transportation anteriorad to the eye and brain area via the internal carotids and posteriorad to the kidneys by way of the renal arteries.

SUMMARY AND CONCLUSIONS

1. Ten strains of the genus Cytophaga of the Order Myxobacteriales, and five strains of the genus Pseudomonas, were injected into the kidneys, the gill filaments, ophthalmic, brain, and peritoneal cavity areas of Rainbow trout and Cutthroat trout.

2. Infections were initiated in all organs injected by several strains of Cytophaga and Pseudomonas. Pathological conditions were similar to those found under natural conditions.

3. Strains of the genus Cytophaga were demonstrated in cartilage surrounding the brain. A possible correlation exists between cartilage-reduction and brain damage.

4. A new pathological condition of the kidney was recorded in the study. This condition existed as crystalline formation in kidney uriniferous tubules.

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